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Incorporating Genetic Sampling in Long-Term Monitoring and Adaptive Management in the San Diego County Management Strategic Plan Area, Southern California

Open-File Report 2017–1061

U.S. Department of the Interior
U.S. Geological Survey

Cover:
Left panel:
Photograph showing a small tail clip being collected for genetic analysis from a blunt-nosed leopard lizard (*Gambelia sila*) captured in the San Joaquin Valley, California. After it is measured and weighed, the lizard is released where it was captured. Photograph by Dustin Wood, U.S. Geological Survey.

Right panel:
Photograph showing DNA being extracted from plant leaf tissues. Photograph by Elizabeth Milano, U.S. Geological Survey.

Incorporating Genetic Sampling in Long-Term Monitoring and Adaptive Management in the San Diego County Management Strategic Plan Area, Southern California

By Amy G. Vandergast

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RYAN K. ZINKE, Secretary

U.S. Geological Survey
William H. Werkheiser, Acting Director

U.S. Geological Survey, Reston, Virginia: 2017

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
kilometer (km)	0.5400	mile, nautical (nmi)
meter (m)	1.094	yard (yd)
	Area	
square meter (m^2)	0.0002471	acre
square meter (m^2)	10.76	square foot (ft^2)

Abbreviations

cpDNA	chloroplast deoxyribonucleic acid
DNA	deoxyribonucleic acid
eDNA	environmental deoxyribonucleic acid
MSPA	San Diego County Management Strategic Plan Area
mtDNA	mitochondrial deoxyribonucleic acid
RAD-seq	restriction site associated DNA sequencing
RNA	ribonucleic acid
SNP	single nucleotide polymorphism
RNA-seq	RNA sequencing
SSR	simple sequence repeat
STR	short tandem repeat

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Abstract

Habitat and species conservation plans usually rely on monitoring to assess progress towards conservation goals. Southern California, USA, is a hotspot of biodiversity and home to many federally endangered and threatened species. Here, several regional multi-species conservation plans have been implemented to balance development and conservation goals, including in San Diego County. In the San Diego County Management Strategic Plan Area (MSPA), a monitoring framework for the preserve system has been developed with a focus on species monitoring, vegetation monitoring, threats monitoring and abiotic monitoring. Genetic sampling over time (genetic monitoring) has proven useful in gathering species presence and abundance data and detecting population trends, particularly related to species and threats monitoring objectives. This report reviews genetic concepts and techniques of genetics that relate to monitoring goals and outlines components of a genetic monitoring scheme that could be applied in San Diego or in other monitoring frameworks throughout the Nation.

Introduction

The major goals of monitoring for conservation management include understanding the trajectories of species and, at a broader scale, species assemblages or communities, in order to detect and mitigate for the unintended consequences of anthropogenic changes, and track the effects of management actions (Noss, 1990; Block and others, 2001; Thompson, 2013). At the regional level of the San Diego County Management Strategic Plan Area (MSPA), a monitoring framework for the preserve system has been developed with a focus on four major areas: (1) species monitoring, (2) vegetation monitoring, (3) threats monitoring and (4) abiotic monitoring (San Diego Management and Monitoring Program, 2012). Species monitoring addresses basic information needs—where individuals are found (distribution); how many are present (abundance); are they able to survive and reproduce (population dynamics, vital rates); what factors or threats promote or, conversely, impede population growth and persistence (habitat and threat associations); as well as the effects of management actions on these factors (Yoccoz and others, 2001; Stem and others, 2005). Although monitoring programs typically use ecological field research techniques applied over time to gather appropriate species data and detect trends (Elzinga and others, 2009), genetic sampling over time also provides important information to support monitoring metrics and objectives (Noss, 1990; Schwartz and others, 2007).

Genetic diversity provides the raw material for **selection** and diversification and is intimately tied to population size and connectivity, and, thus, to persistence and **adaptive** potential (Words and phrases are presented in bold font the first time they appear in the text and are defined in the "Glossary"). Genetic sampling of many individuals within and among aggregations or **populations** can be used to directly estimate the amount and distribution of genetic diversity (both neutral and adaptive) and indirectly estimate connectivity among localities. This allows one to identify populations with low genetic diversity and small sizes, and populations that are isolated because natural **dispersal** and **migration** patterns are inhibited. These populations may be more vulnerable to local extinction without management action. Collecting genetic data at a single time point can create a "snapshot" of population **genetic structure** and diversity, with many recent examples pertaining to species in the MSPA (table 1). Some types of genetic data can provide an efficient means of estimating important population parameters including **gene flow, breeding population size,** and genetic diversity. These parameters are important for evaluating the health and connectedness of populations of rare plants and animals in managed landscapes. However, given that populations are dynamic, parameter estimates also are likely to change over time, particularly in rapidly changing environments (Vandergast and others, 2016).

What is Genetic Monitoring?

Genetic monitoring tracks changes in the amount and distribution of genetic diversity across populations over time using **neutral genetic markers**, and follows adaptive genetic responses to changing environmental conditions. Species-focused genetic monitoring has been used to detect and quantify changes in gene flow, breeding population size, and genetic diversity over time, and evaluate the effects of ongoing management against baseline standards. Additionally, **genetic mark-recapture** techniques and DNA-based techniques for identifying species have been used to estimate other population parameters in monitoring efforts, including census size, abundance, and distribution, and may be particularly useful when species or individuals are cryptic or difficult to track using other techniques. In this report, I review the different types of information provided by genetic monitoring, the applicability of this information to management, the available tools for genetic sampling, and considerations for choosing appropriate sampling designs and markers for monitoring given recent advances and changes in genetic and genomic data-collection techniques.

Brief Review of Current Genetic Data Collection and Analysis Methods

Before discussing the application of genetic data to monitoring, it may be useful to briefly review current genetic markers and data-collection techniques. Although there are various different genetic markers that have been favored over the years (see table 1 for some examples and descriptions), two of the most common markers presently (2017) used in population genetic inquiries are **microsatellite loci** and **SNPs** (single nucleotide polymorphisms). For questions of species identification and presence, where identification of unique individuals and population diversity statistics are not needed, mitochondrial DNA markers are often used for animals, whereas chloroplast or nuclear ribosomal **genes** are often used for plants.

Table 1. Overview of different genetic markers.

[See also National Human Genome Research Institute Talking Glossary of Genetic Terms, available at https://www.genome.gov/glossary/. **Abbreviations:** AFLPs, amplified fragment length polymorphisms; DNA, deoxyribonucleic acid; cpDNA, chloroplast DNA; eDNA, environmental DNA; mtDNA, mitochondrial DNA; RAD-seq, restriction site associated DNA sequencing; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; STR, short tandem repeat]

Marker type	Description	Sample quality/quantity	Recommended for new population-level monitoring studies?
Allozymes/isozymes	One of the first marker types used for population genetic analyses. Enzymes with allelic variants distinguishable based on gel electrophoresis. Typical marker sets are comprised of 10–20 allozyme loci. Markers tend to be fairly conserved (low diversity).	High; need large quantities of fresh tissues to assay proteins.	No. Low diversity (few alleles at most loci), difficult to run more than 10–20 markers because of high amounts of fresh tissue needed for analysis. Chemicals used for gel staining can be very toxic.
Organellular DNA (mitochondrial and chloroplast DNA) and nuclear intron DNA sequencing	Direct sequencing of a gene or gene region. Mitochondrial gene regions have been commonly sequenced for animals and chloroplast gene regions for plants, along with nuclear intron regions for both plants and animals. MtDNA and cpDNA each represent a single linked locus, even if multiple gene regions are sequenced within each. Development of primers is required to sequence the region of interest. These markers traditionally have been more useful for understanding phylogenetic/systematic relationships among species or populations. Typically less than 10 independent markers are examined in traditional sanger sequencing studies.	Low to medium, depending on specific markers. Organellular DNA tends to occur in high copy number, so that smaller amounts of starting material may be acceptable.	Yes for species identification applications (eDNA, hair/fecal samples, etc.). No for detecting population-level trends. Although direct gene sequencing is useful for understanding evolutionary relationships among lineages, population genetic studies based on these markers may suffer from low power because of low number of markers. Data from reduced representation library sequencing can be analyzed as short sequence reads rather than as individual, independent SNP sites. These types of methods may replace direct sanger sequencing for generating sequence data for phylogenetics.
AFLPs	ALFPs. **Restriction enzymes** are used to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is selected to be amplified using primers complementary to the adaptor sequence, the restriction site sequence, and a few nucleotides inside the restriction site fragments. The amplified fragments are separated and visualized with fluorescence using automated capillary sequencing. Resulting data are not scored as length polymorphisms, but as presence/absence polymorphisms.	High	No. Although AFLPs allow many presumptive loci to be examined (typically hundreds to low thousands) inexpensively, only the dominant alleles are directly visualized. Heterozygotes must be inferred under some sort of model, such as assuming Hardy Weinberg equilibrium, or many standard population genetic analyses cannot be performed. These markers are less useful for understanding patterns of heterozygosity across or within populations.

Marker type	Description	Sample quality/quantity	Recommended for new population-level monitoring studies?
Microsatellites/SSR/ STRs	A microsatellite is a tract of repetitive DNA sequence (the repeated motif is usually 2–4 bases long). These occur at thousands of locations within an organism's genome, and additionally have a higher mutation rate than other areas of DNA leading to high diversity. Typically, datasets will include 10–20 loci. Marker development includes identifying repeat regions from shotgun or RAD-seq sequence data; primers are then designed and tested for variability and amplification consistency before markers can be used in a full study. Markers usually are species-specific, although some will cross-amplify across closely related species.	Medium. Low-quality environmental samples should be run multiple times. Allelic drop out can be higher in more degraded samples.	Yes. High variability makes these markers useful for investigating population-level questions, identifying individuals by genotype and particularly working with samples obtained non-invasively that typically contain less DNA that is more degraded than when fresh tissues can be obtained. However, simulations have shown that in detecting trends over time, such as decreasing breeding population size (N_b) over time or migration rates in the range of demographic connectivity (greater than 10 percent), power is limited at 20 microsatellites. Larger numbers of markers (thousands) can increase power, precision, and accuracy.
SNPs	SNPs are nucleotide variants (A, C, T, G) that occur in a particular base position in the genome. For example, at a specific base position, the base C appears in most individuals, but in a minority of individuals, the position is occupied by base A, and heterozygous individuals have both a C and an A. There is a SNP at this specific base position, and the two possible nucleotide variations—C or A—are alleles for this base position. "Next generation sequencing" or "massively parallel sequencing" that sequences millions of DNA molecules simultaneously is commonly applied to discover SNP loci. Because genomes are very large, methods to reduce or select the amount of genomic DNA from an individual are applied—for example, "RAD-seq." Once loci are detected, capture methods can be used to sequence additional individuals at the same loci over time.	Variable	Yes. Costs for RAD-seq sequencing can be similar to microsatellite design, testing, and analyses. This technique requires relatively high quality and quantity of genomic DNA to start with. Significant bioinformatic processing of resulting datasets is required. These methods are becoming more commonly applied to non-model organisms for population genetic analyses, and methods and biases are beginning to be explored. Loci are anonymous, unless their positions can be mapped to an existing genome. Association studies can be used to detect putatively adaptive loci.

Choosing Genetic Markers

There are three major considerations and constraints in choosing among marker types and determining how many markers are needed for population genetic monitoring: (1) the **effect size** to be detected, (2) the cost of data collection, and (3) the amount and quality of tissues for **DNA extraction**. **Power** and **precision** in estimating genetic parameters are related to the number and diversity of **independent markers** that can be examined and the number of individuals that can be surveyed. Generally, power and precision increase as the number of independent markers and individuals sampled increase (Paetkau and others, 2004; Hoban and others, 2013). Constraints on datasets are related to the costs to sample individuals and to develop and survey genetic markers. Advances in **gene sequencing** technologies over the past decade, including **high throughput** (or "next generation") **sequencing**, have greatly increased the number of genetic markers that can be surveyed, as they have greatly decreased the per-**base** costs of generating sequence data (National Human Genome Research Institute, 2017, figs. 1 and 2). Costs to produce SNP datasets with thousands of individual SNPs are currently on par with the costs to develop and survey dozens of microsatellite loci only a decade ago, and costs continue to decrease. Because of this, genetic datasets are shifting to include larger and larger numbers of markers, and adaptive as well as selectively neutral genetic diversity can be more readily surveyed (see table 2 for types of high throughput sequencing). The amount and quality of the DNA sampled also will play a role in choosing the most appropriate marker type. For example, fecal samples and older museum samples typically contain more degraded template DNA than freshly obtained blood or tissue (Wandeler and others, 2003; Schwartz and others, 2007). This may lead to problems with **allelic dropout**, requiring further steps to verify **genotypes** and remove errors (Miller and others, 2002). Some next generation sequencing protocols, such as whole genome sequencing, RNA sequencing (RNA-seq), and to some extent RAD-seq (Restriction site Associated DNA Sequencing; see table 2 for descriptions) typically require much larger amounts of good quality DNA and can perform poorly with degraded samples (Andrews and others, 2016). However, newer RAD-seq protocols report better results with samples of varying quality (Ali and others, 2016). Other methods such as targeted sequencing using capture probes (table 2) may be more robust to sample degradation than other high-throughput sequencing methods (Bi and others, 2013).

Neutral and Adaptive Markers

Not all genetic markers are equally applicable to estimating every population parameter. One of the most important considerations is whether the genetic markers behave as selectively neutral. Monitoring of population demographic processes and parameters such as **effective population size** (**N_e**) and gene flow require the use of selectively neutral genetic markers. Changes in **allele** frequencies and inheritance patterns in neutral markers are assumed to be driven largely by demographic processes (Wright, 1931; Hartl and Clark, 1989). However, when markers are within or tightly **linked** to genes of adaptive significance, allelic distribution patterns likely indicate **selection** (Lewontin, 1964). Monitoring for **local adaptation** requires analysis of the genes or closely linked loci (loci located on the chromosome in close physical proximity) to the genes that underlie adaptive traits. High throughput sequencing techniques offer opportunities to simultaneously survey and identify neutral and adaptive variation (Allendorf and others, 2010). Thus, applying high throughput sequencing in genetic monitoring can allow an understanding of gene flow and breeding population size as well as insight into local adaptation and identification of genes important in adaptive traits.

Table 2. Types of high-throughput sequencing techniques that can produce single nucleotide polymorphisms, short sequence reads, or assembled genomes.

[Information summarized from Allendorf and others, 2010; Andrews and others, 2016; Oyler-McCance and others, 2016. **Abbreviations:** DNA, deoxyribonucleic acid; RAD-seq, Restriction site Associated DNA Sequencing; RNA, ribonucleic acid; RNA-seq, RNA sequencing; SNP, single nucleotide polymorphism]

Type	Name	Description	Sample quality	Relative cost per sample	Applications
Reduced, anonymous	RAD-seq	RAD-seq is a group of restriction site associated DNA sequencing methods that require no a priori genomic resources to apply. RAD-seq techniques begin by digesting DNA with one or more restriction enzymes then add sequencing adaptors and barcodes, which are used to identify individual samples that are sequenced together (multiplexed). RAD-seq protocols also reduce and (or) select the sizes of DNA fragments that are optimal for next-generation sequencing. Typically, thousands to tens of thousands of markers are recovered, although even larger datasets are possible.	Variable	Low	Estimating gene flow, effective population size (N_e), individual identification, constructing pedigrees. Variable SNP loci identified through anonymous RAD-seq can then be used to design targeted capture for future monitoring. Association studies to detect adaptation.
Transcriptome	RNA-seq	The transcriptome is the set of all messenger RNA in a cell or sample and represents the genes that are being actively expressed at a given time. Transcriptome sequencing, or RNA-seq, is used to analyze the continually changing cellular transcriptome. RNA-seq facilitates the ability to look at mutations/SNPs and changes in gene expression, among other uses. A transcriptome can be assembled de novo from RNA-seq short reads, or mapped to an existing genome. SNPs identified through RNA-seq are associated with expressed genes. RNA-seq can be combined with RAD-seq to map anonymous SNPs in the absence of a reference genome.	High, plus RNA must be preserved	Low	Gene expression. When assembled genomes are lacking, anonymous SNPs (from RAD-seq) can be mapped against the transcriptome to assess functionality.

Type	Name	Description	Sample quality	Relative cost per sample	Applications
Reduced, targeted	Targeted capture	Targeted capture methods use custom DNA "probes" or "baits" that are complementary to the target region of interest and bind to that target region in the sample DNA. These captured fragments are isolated from the DNA, amplified using a polymerase chain reaction, and then sequenced on a high-throughput sequencer.	Variable	Higher than anonymous RAD-seq	Useful for repeated genetic monitoring efforts because they consistently target the same set of loci and capture sets can be designed to incorporate both presumptive adaptive and neutral loci. Lower quality samples (for example, museum) can be used.
Whole genome	Whole genome sequencing and resequencing	Resequencing gathers whole genome sequences for multiple individuals in a sample. To assemble a reference genome, genomic DNA is fragmented and short reads (50–300 base pairs) are sequenced. These are aligned to reconstruct the target DNA sequence. Newer sequencing platforms allow for much longer reads (10–15 kilobase pairs, increasing the accuracy of genome assemblies. With an assembled and annotated genome or linkage map, variation at coding and non-coding regions can be surveyed, and methods such as determining inbreeding levels through runs of homozygosity throughout the genome could be applied. Adaptive differences at few loci of large effect can be more easily detected than with methods such as RAD-seq that subsample the genome prior to sequencing.	High quality and quantity	High	Linkage mapping, runs of homozygosity, genome structure (for example, chromosomal inversions), selective sweeps. For monitoring neutral population genetic parameters (N_e, gene flow) that can be estimated well with hundreds to thousands of neutral markers, the expense for whole-genome (re)sequencing may excessive. As costs continue to decrease, resequencing may become more common.

Population Genetic Parameters Useful for Monitoring

The relationship between specific species and threats monitoring goals and population genetic parameters is summarized in figure 1. Depending on how they are analyzed, genetic data can be informative over both evolutionary and ecological time scales (Bohonak and Vandergast, 2011; Rissler, 2016). Analyses that rely on the genetic distance among alleles or haplotypes (variants of genes or gene regions) typically represent the time scales over which gene mutations occur and accumulate (evolutionary time), whereas analyses that rely on changes in allele frequencies, **linkage disequilibrium**, and tracing **pedigrees** with multi-locus genotypes can represent changes across shorter time scales of only one or a few generations (Pritchard and others, 2000; Waples and Do, 2010; Bohonak and Vandergast, 2011; Moore and others, 2014; Wang, 2014). For monitoring efforts, parameters and estimation techniques that represent these ecological time scales may be useful because they are more likely to detect changes over monitoring time frames.

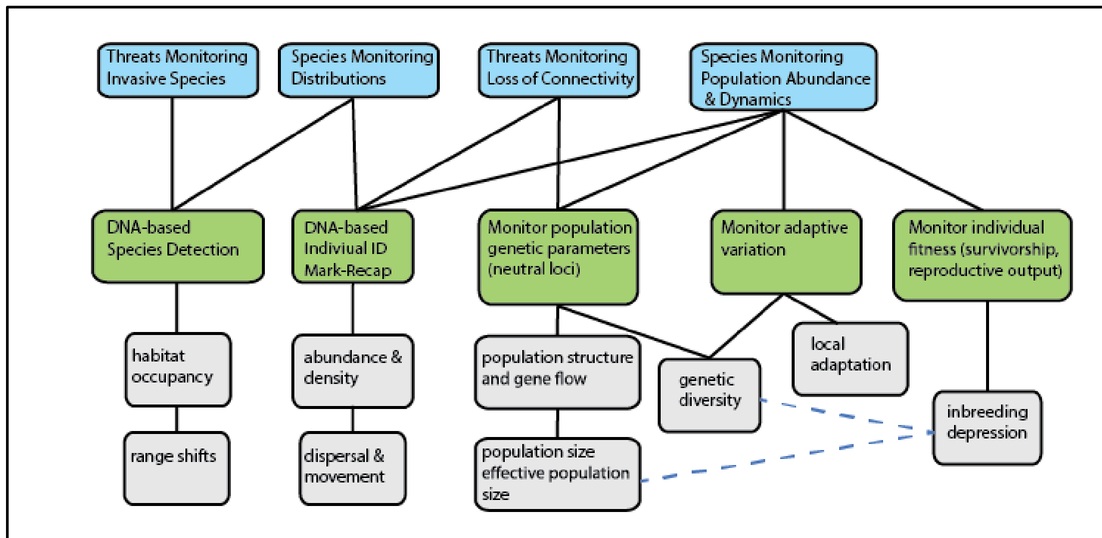

Figure 1. Schematic showing relevance of genetic techniques to monitoring metrics. Monitoring metrics (top row of boxes) developed for the San Diego County Management Strategic Plan Area can be informed by genetic monitoring approaches (second row of boxes) and population parameters (bottom rows of boxes) can be estimated with each approach. Dashed lines indicate that estimating inbreeding depression is particularly informative when done in tandem with estimating diversity and effective population size.

Among Populations—Genetic Structure and Gene Flow

We can measure the amount and partitioning of genetic diversity within and between populations. The partitioning of genetic diversity among populations (or subpopulations) is referred to as **genetic differentiation** or genetic structure and is inversely related to and provides an indirect measure of the amount of genetic exchange or gene flow among locations. Gene flow differs from dispersal or movement in that it requires successful migration and breeding, and so indicates intergenerational time scales and is typically correlated with dispersal ability (Bohonak, 1999). Monitoring population genetic structure and (or) estimating gene flow over multiple generations can alert conservation managers to changes in habitat connectivity among sites and could be particularly important in regions where habitat loss or disturbance are suspected to affect individual movement rates. For example, genetic analysis of coastal cactus wrens (*Campylorhynchus brunneicapillus*) throughout southern California indicated that aggregations of wrens were highly genetically differentiated, concordant with loss and fragmentation of habitat throughout their range (Barr and others, 2015). Conversely, California gnatcatchers (*Polioptila californica*) occupying a similar range comprised a single genetic population (Vandergast and others, 2014), highlighting differences in effective dispersal in fragmented landscapes between these two rare species, and supporting different management and monitoring strategies in each case.

Genetic differentiation among populations can accumulate rapidly when population sizes are small. Additionally, the direction of the change can make a difference in the time scale of detection. For example, simulations showed that signatures of fragmentation can take more generations to become genetically detectable than signatures of increased gene flow among previously differentiated sites, and that individual-based genetic differentiation metrics are more informative than population-level metrics (Landguth and others, 2010). Detectability also may depend on the number of markers. Power and precision can increase when more unlinked markers are surveyed. The accuracy of parameter estimation theoretically should be improved when more loci are examined because it lessens the effect of individual loci that may differ because of stochastic gene processes or selection, and facilitates the identification and exclusion of loci under selection (Allendorf and others, 2010).

Measures of genetic differentiation can be compared over time to determine trends (increasing or decreasing) or can be compared to a predetermined threshold. In theoretical models of randomly mating populations, **genetic drift** counteracts gene flow at a level of one effective migrant per generation or less (Mills and Allendorf, 1996). Below this threshold, populations will become genetically independent and genetic differences will accumulate. Managers also may be interested in determining whether movement rates reach **demographic connectivity**, where the growth of a population is affected by the level of dispersal or immigration into the population. This measure is important for population persistence, such as rescuing small populations from extinction, and conferring stability in **metapopulations** with high extinction and colonization rates (Lowe and Allendorf, 2010). Demographic connectivity thresholds occur at much higher movement rates than genetic connectivity. Modeling by Hastings (1993), suggested that populations tend to become linked demographically at moderate migration rates of about 10 percent, whereas genetic linkage occurs at migration rates of greater than one migrant per generation.

Gene flow traditionally has been estimated indirectly, through approximating the amount of **genetic differentiation** among populations ($F_{ST;}$ Slatkin, 1985). More recent Bayesian and likelihood approaches have been developed that estimate migration rates and population assignments based on individual genotypes (Wilson and Rannala, 2003), or parentage (Wang, 2014). Limitations to these methods have been noted, and accurately estimating levels of gene flow approaching or exceeding a 10-percent threshold (for demographic connectivity) can be difficult (Faubet and others, 2007; Meirmans, 2014). Genomic data collection techniques show promise in these areas, as thousands to tens of thousands of SNPs may increase the accuracy of estimating higher gene flow rates (Andrews and others, 2016).

Within Population—Effective Population Size, Genetic Diversity, and Inbreeding

The census size of a population (N) is related to population demography and is influenced by factors such as birth and death rates, competition, and predation (Sutherland, 1996). Monitoring of population abundance and density are instrumental to population monitoring protocols, and estimation is regularly implemented through techniques such as count and transect surveys, camera trapping, and occupancy modeling. Likewise, the effective population size (N_e) also is an important population parameter that should be monitored over time. It influences the loss of genetic variation over time (genetic drift), the rate of accumulation of mutations (genetic diversity), and the efficiency of natural selection. N_e is defined as the size of an ideal population (Wright-Fisher model; Wright, 1938) with the same rate of change as the population under consideration. N_e approximates the number of breeding individuals in a population producing offspring that live to reproductive maturity. N_e usually is smaller than the population census size because successful breeding rarely is equal among all individuals, owing to unequal sex ratios, unequal mating success, unequal **fitness** of offspring, and other factors. When N_e is very small, it can be a warning sign that populations are decreasing and are vulnerable to extinction. For example, recent genetic work on southern California pumas indicated that the Peninsular Range population had a N_e of 24, and that the Santa Ana Mountains had a N_e of 5, with little evidence of recent movement between these groups (Ernest and others, 2014).

A question of interest for conservation is how big does N_e have to be to reduce loss of genetic variation? Based on information from livestock breeding and experimentation with fruit flies, several thresholds have been proposed. N_e greater than 50 is necessary for avoiding inbreeding depression in the short term, and at $N_e = 500$, genetic diversity should reach an equilibrium where the gain from mutation is equal to that lost because of genetic drift (Frankham, 1996). Recently, based on a review of empirical studies, Frankham and others (2014) suggested thresholds of $N_e = 100$ to avoid inbreeding depression and $N_e = 1{,}000$ to retain adaptive potential in wild populations. These thresholds apply to closed populations (with no immigration). Increasing migration and gene flow, even by small amounts, can significantly improve local genetic diversity and increase N_e. In metapopulation systems with sites connected by gene flow, local estimates of N_e may be more indicative of the number of breeding individuals in the larger metapopulation, rather than the number of effective breeders at that site (Waples and England, 2011).

N_e can be estimated from genetic data in different ways. Temporal estimators require sampling a site at two points in time (Jorde and Ryman, 2007). Single sample estimators, such as the **linkage disequilibrium** method (Waples and Do, 2008), estimate N_e from a single sampling time point. Single sample estimates are indicative of the generation before sampling occurred (and may be influenced slightly by previous generations, as there is some background linkage disequilibrium that remains from recent previous generations). Temporal estimates represent the harmonic mean of N_e across that time period (Waples, 2005). An estimate of N_e from a single age cohort (for example, nestlings, tadpoles, fish fry) is defined as the number of breeders (N_b) in a site (Waples, 2005). N_b may be a preferable monitoring metric to N_e, given that true N_e is difficult to accurately estimate in **age structured populations** and with overlapping generations. Because N_b is a measure of population specific reproductive output, it may have a more direct relationship to current habitat quality or other site-specific effects that may limit population growth and persistence. For example, Whiteley and others (2015) found a high correlation between local patch size and N_b in a stream salmonid. One final consideration is that the precision of estimating N_e and N_b using genetic methods decreases as these parameter values increase; therefore, it is difficult to accurately measure very large effective or breeding population sizes (Waples and Do, 2010).

Genetic diversity is influenced by population size and provides the raw materials for adaptation; therefore, monitoring changes in genetic diversity metrics also can provide information about population size and evolutionary potential. Genetic diversity often is quantified in terms of the number of alleles (A) and **heterozygosity** (H). In the case of a decrease in population size, the number of alleles is expected to decrease more rapidly than heterozygosity because rare alleles typically are lost first. Although rare alleles are weighted equally in contribution to the number of alleles, their contribution to heterozygosity is much less than high-frequency alleles (Allendorf, 1986; Leberg, 2002). Heterozygosity at neutral loci decreases at a rate of one divided by $2N_e$ per generation in the absence of gene flow (Lacy, 1987).

Allele frequencies also can change rapidly in decreasing populations, and so changes in allele frequency over time ($F_{TEMPORAL}$) may indicate changes in population size (Richards and Leberg, 1996). In fact, temporal methods of estimating N_e rely on this relationship (for example, Jorde and Ryman, 2007). Because steep decreases leave a distinct genetic signature, genetic erosion can be detected and signals may be detectable for several generations post **genetic bottleneck** (Cornuet and Luikart, 1996; Garza and Williamson, 2001). Recent population growth in a closed population (growth through increased reproduction and survivorship compared to growth through immigration and gene flow) may be more difficult to detect with diversity estimates, such as monitoring the number of alleles. Although immigration can increase diversity in the receiving population by introducing alleles that were already present in the source population, when a population is closed to immigration, new diversity arises only through mutation. Per-site mutation rates are very low (about 1×10^{-3} - 1×10^{-6} per generation) and have little effect on genetic diversity over short time periods (Lacy, 1987). In such cases, estimating changes in N_b, or census size (through genetic marking or field techniques) may provide more insight.

Small populations likely will have higher levels of inbreeding than larger ones. Breeding between relatives lowers heterozygosity and can reduce the **fitness** of offspring (inbreeding depression), which can then adversely affect population growth rates (Crnokrak and Roff, 1999). Although levels of inbreeding can be estimated using genetic information or through pedigrees (inbreeding coefficient, F), determining fitness consequences also requires gathering data on individual fitness (Crnokrak and Roff, 1999). Estimates of the inbreeding coefficient based on heterozygosity (F_H) have been shown to be imprecise when based on a few markers, and so calculating F using pedigrees has been advocated (Pemberton, 2004). However, pedigrees can rarely be calculated for more than a few generations, and more recent work has shown that F_H can reach higher levels of precision than pedigree-based estimates when the number of markers is increased to the thousands (Kardos and others, 2015). At even higher numbers of markers (hundreds of thousands) and with an available reference genome for alignment, inbreeding can be estimated even more precisely from mapping regions of high homozygosity (runs of homozygosity), in which the length of the runs is correlated with the level of inbreeding (Kardos and others, 2015).

Determining and Monitoring Adaptive Variation

One challenge in species management is determining whether population augmentation is warranted as a management strategy in small or decreasing populations. If so, should new occurrences be started through translocation, and what is the best strategy to identify which source populations should be used? Genetic monitoring based on neutral genetic markers can help to determine the population size, diversity, and connectedness across the landscape, and help point to the most genetically similar populations on the landscape. Diversity can be important in transplanted populations. For example, when genetic diversity and effective population size in transplanted populations are low, this can result in reduced fitness and population viability, and ultimately failure of the transplanted population (Ellstrand and Elam, 1993; Newman and Pilson, 1997; Schmidt and Jensen, 2000). For example, Helenurm and Parsons (1997) found that populations of salt marsh bird's-beak (*Cordylanthus maritimus* ssp. *maritimus*) that failed to thrive when reintroduced to Sweetwater Marsh contained almost no genetic variation, and Williams (2001) found a correlation between fitness and genetic diversity of transplanted eelgrass populations in San Diego Bay. Conversely, concerns of outbreeding depression may restrict the use of managed gene flow among isolated small populations, although this may only be of concern in extreme cases (Frankham and others, 2011). Outbreeding depression can result from either chromosomal or genic incompatibilities between distantly related groups (for example, chromosomal differences, intrinsic outbreeding depression) or reduced adaptation to local environmental conditions (extrinsic outbreeding depression; Edmands, 2007). In the latter case, transplanted individuals and their offspring may have reduced fitness in that local environment. However, selection works most effectively in large populations with high diversity, whereas in very small populations, it is inefficient in countering drift (Lacy 1987). Therefore, selection may be of little effect in small and decreasing populations.

Instead of using variability in neutral loci as a proxy for adaptive potential, if the gene(s) underlying adaptive traits can be identified, these can be directly monitored to track responses to environmental change. Reciprocal transplant experiments can show large survival and reproductive differences among individuals (Hereford, 2009), and genomic association (Hirschhorn and Daly, 2005) and clinal analyses have been used to determine underlying traits of the genes (Hansen and others, 2012). Methods to locate genes under selection are relatively novel at the time of this report and may be more challenging to apply to non-model organisms than approaches involving neutral genetic variation. Genomic level inquiries of many SNPs (on the order of hundreds of thousands), **transcriptome sequencing**, and whole genome sequencing approaches have allowed researchers to locate putatively adaptive loci in non-model organisms through association studies and outlier tests (Hirschhorn and Daly, 2005; Narum and Hess, 2011).

Quantitative traits (**phenotypically** variable and measurable traits) also can be tracked to provide information on adaptive response. However, the technique has not been applied widely to natural populations because the heritable component of quantitative genetic variation can only be scored when the relatedness among a substantial number of individuals in a population is known (this can be determined using crossing experiments in plants for example).

A set of adaptive markers or traits selected for monitoring should include loci that contribute to a substantial part of the genetic variance in a trait within and between populations and that undergo a substantial change in allele frequency correlated with environmental change (Hansen and others, 2012). If such loci can be identified, there is the potential to monitor allelic changes to understand how selection acts on a population under changing environmental conditions and to identify and preserve particular genetic and phenotypic variation that is deemed important for population persistence. For example, in Tasmanian devils (*Sarcophilus harrisii*), resistance to the transmissible cancer causing facial tumors has been detected and is associated with particular gene variants that map to chromosomal regions associated with immune function (Epstein and others, 2016). Individuals containing these variants may be selected for translocation to populations in which the disease is prevalent. In a different example, Shryock and others (2017) mapped habitat features associated with putative adaptive variation in two Mojave Desert shrub species to designate climatic seed transfer zones for restoration.

In addition to tracking genetic variation at adaptive genes, **gene expression** levels also can be monitored in populations, at panels of pre-determined genes or through whole transcriptome sequencing approaches (table 2). This is useful when expression levels at known loci have been related to underlying factors of interest such as health or response to disease exposure. For example, gene expression profiles at a panel of immune response and physiological-defense associated genes are being assessed in wild and captive desert tortoises to better understand relationships between individual health and habitat (Bowen and others, 2015).

Detection—Environmental DNA and Genetic Mark-Recapture

Genetic methods are being used increasingly to determine species presence, and to identify individuals in mark-recapture studies to estimate density, abundance, movement, and dispersal. DNA-based species detection and individual identification techniques may be particularly useful in cases where other field monitoring methods have low probabilities of detection, when DNA sample collection and laboratory analyses can be done more efficiently and cost effectively than direct observation methods (for example, trapping), or when genetic sampling techniques are less invasive to sensitive species or habitats than other direct monitoring techniques. Species-level and individual-level identification from environmental samples require unique marker and protocol development and testing as well as establishing detection thresholds prior to implementation. Protocol development should be factored into the costs and time frame for establishing any environmental DNA monitoring program.

Species-level diagnostic markers (typically mitochondrial [mtDNA] or chloroplast [cpDNA] markers) can be amplified and sequenced from samples that are not easily identified morphologically (for example, fairy shrimp cysts; Vandergast and others, 2009) and from mixed environmental samples (eDNA—small amounts of DNA available in occupied water or soil [Rees and others, 2014; Fahner and others, 2016]). For example, in the MSPA, presumed badger sign identified by detection dogs was confirmed by amplification of badger mtDNA to confirm presence of badgers and exclude other animals (Brehme and others, 2012). This type of monitoring information can be useful in determining species presence, and in estimating site occupancy or changes in geographical ranges of species of interest. Environmental DNA approaches also may be useful in monitoring invasive species and have been applied to monitor the spread of disease (Kirshtein and others, 2007).

Similarly, DNA from scat and hair samples can be used to identify individuals. Typically, these types of studies have used a suite of species-specific variable markers, such as microsatellite markers, that when amplified together provide high probability of individual identification (Waits and Paetkau, 2005). Amplification and accurate genotyping sometimes can be problematic because environmental samples tend to be subject to more DNA degradation, leading to allelic drop out (where one or more alleles does not amplify), which can lead to misidentifications. Typically, samples are subjected to multiple rounds of independent amplification and genotyping to determine scoring error rates (Miller and others, 2002).

When applied in a spatial mark recapture sampling framework, individual **genetic recaptures** can be used to estimate density and abundance, and to track movement of individuals (Mills and others, 2000; Lukacs and Burnham, 2005). The same genetic data used for individual identification for mark recapture purposes also can be used to calculate population genetic parameters, simultaneously providing multiple levels of information in monitoring programs. Non-invasive DNA sampling is being used in long-term monitoring of brown and black bear populations (Kendall and others, 2009; De Barba and others, 2010; Sawaya and others, 2012) and has been used in the MSPA to study southern mule deer movement (Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016).

Incorporating Genetic Monitoring into a Strategic Monitoring Plan

Regular monitoring efforts will yield important and sometimes unanticipated insights. For example, long-term pitfall sampling conducted by U.S. Geological Survey across the MSPA preserve system allowed investigators to compare pre- and post-fire species assemblages to assess recovery after the catastrophic wildfires of 2003 and 2007 (Rochester and others, 2010). The same is likely to be true of genetic monitoring efforts, regardless of their initial objectives (Vandergast and others, 2016). Some useful components of a genetic monitoring scheme (adapted from Schwartz and others, 2007) are summarized as follows.

1. **Identify the objectives.** As with other types of ecological data collection for monitoring, it is important to identify objectives and set benchmarks or criteria for identifying biologically significant change. Single time point genetic surveys typically are used to address baseline objectives, such as estimating the number of independent gene pools or populations across a species range (Barr and others, 2015; Wood and others, 2016). Results from initial genetic surveys may help focus on more specific monitoring objectives by indicating potential concerns for long-term population persistence within the plan area (see table 3 for management objectives and related genetic monitoring techniques). For example, initial genetic surveys of coastal cactus wrens described high genetic differentiation among populations and low genetic diversity within populations, with patterns related to the availability of habitat (Barr and others, 2015). Management actions are underway to expand and connect habitat fragments to increase N_e and reduce the potential for inbreeding. Future genetic monitoring efforts could focus on resurveying genetic connectivity between reconnected sites. Defining very specific objectives (for example, estimate N_b with a coefficient of variance less than 0.5; detect immigrants with 95-percent probability) can help to ensure that the most appropriate genetic analysis tools and study design are selected at the outset.

Table 3. San Diego County Management Strategic Plan objectives for monitored species and related genetic questions and potential analysis techniques.

[**Abbreviations:** Nb, breeding population size; Ne, effective population size; SNP, single nucleotide polymorphism]

Management Strategic Plan objectives	Genetic questions	Markers and analyses
Seed banking, enhancing an existing occurrence, establishing new occurrences, abundance and distribution within a population.	Bank from many or few locations? What is the genetic similarity among individuals at different existing occurrences? Is the distribution of genetic diversity correlated with geographic (distance) or habitat variables? What is the relative fitness of crossed compared to single source offspring?	SNPs, screen for neutral and adaptive variation, measure fitness.
Establish self-sustaining populations.	How large is the population? How much genetic diversity does it contain relative to other populations? Is it connected by gene flow to other populations? Is the population growing, shrinking, or stable over time?	SNPs or microsatellites; monitor N_b, gene flow rates.
Monitor reproduction.	How many individuals contribute to the next generation? What is the genetic relatedness among individuals?	SNPs or microsatellites, pedigree analyses, N_b, possibly inbreeding depression.
Improve connectivity between populations.	What is the functional or genetic connectivity among sites? What environmental factors are associated with or impeding connectivity?	SNPs. Monitor gene flow, genetic - environment association studies.
Maintain and enhance population.	What is the effective population size? What is the level of genetic diversity within the population? Does size fluctuate or is it stable?	SNPs or microsatellites. Monitor for N_e or N_b
Assess baseline data and sex ratio.	What is the abundance and distribution of individuals? How many genetic units are there? Do populations have low diversity or are bottlenecked?	SNPs or microsatellites (if genetic mark recapture), sex specific markers. Genetic clustering/differentiation analyses, estimate genetic diversity, N_e.

2. **Evaluate different sampling and analytical methods.** Once study objectives are identified, there are many considerations in developing the study design. Which method will provide the right type of data? How many markers and individuals should be sampled to provide acceptable power? What type of sample collection is feasible? What experimental design should be applied? For example, in investigating effects of fragmentation, can "control" plots in contiguous habitats be sampled in comparison? What is the appropriate and feasible level of replication? What is the most appropriate sampling scale and spatial configuration for the question? How frequently should sampling occur? Individual-based simulation studies can be helpful in determining the various effects of the number and spatial arrangement of samples, number of markers, and associated power to detect changes or trends (Hoban and others, 2012).
3. **Evaluate and adapt monitoring strategy over time.** Results of monitoring should be evaluated periodically to determine whether the approaches are yielding the desired information and precision needed to inform management. Monitoring plans also should be flexible enough to respond to unanticipated factors. These can include ecological disturbances such as wildfires and droughts, and advances in analytical techniques. In the case of genetics, there have been rapid and major advances in genetic data generation and analysis techniques over the past decade that are likely to continue. Therefore, if a genetic monitoring program will be ongoing over an extended period of time, it is likely that other informative techniques will emerge.
4. **Develop a strategy for sample and data management over time.** Long-term tissue and DNA archiving should be incorporated in plans (Wong and others, 2012). One important consideration is whether the science entity, the management entity, a dedicated repository (such as a museum), or some combination of these should be responsible for long-term sample storage and maintenance. An additional consideration is archiving generated genetic data and analysis results in a format accessible to continued monitoring efforts. National databases exist for some types of raw genetic and genomic sequence data (https://www.ncbi.nlm.nih.gov/), and federal science and funding agencies have mandates to make all data and reports publically accessible (https://www.whitehouse.gov/blog/2013/02/22/expanding-public-access-results-federally-funded-research). One exception is that there are no standardized public repositories for microsatellite data. Because long-term monitoring programs may be developed with multiple science and management entities over time, minimum data archiving and sharing requirements should be established for microsatellite and other molecular studies.

Conclusions

Genetic monitoring has the potential to efficiently address important data needs in species and ecosystem monitoring programs. Genetic and genomic monitoring techniques can help support specific species monitoring objectives (distributions, population abundance, and dynamics of target species) and threats monitoring (detection and dynamics of invasive species, loss of connectivity) in the San Diego County Management Strategic Plan Area. Genetic data collection can be combined with other types of monitoring— such as determining reproduction and survival rates, and understanding habitat associations—to provide important insight into management needs. Baseline genetic information is available or is being gathered for many species in the Management Strategic Plan Area that will facilitate genetic monitoring in the future.

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Glossary

Note: Definitions adapted from National Human Genome Research Institute Talking Glossary of Genetic Terms; https://www.genome.gov/glossary/ and Hartl and Clark, 1989).

Adaptive Referring to a genotype or trait; conferring high relative fitness to individuals possessing it.
Adaptive diversity The diversity of genotypes in a population with potential for future trait evolution in new conditions.
Age Structured Population A population in which the number of individuals differs among different age groups.
Allele One particular form of a gene (diploid organisms have two of each).
Allelic dropout A commonly observed source of missing data in microsatellite genotypes, in which one or both allelic copies at a locus fail to be amplified by the polymerase chain reaction.
Base or base pair A single "position" (or single nucleic acid) on a strand of DNA containing an adenine, cytosine, guanine, or thymine nucleobase. Nuclear DNA is double stranded with complimentary bases or base pairs (guanine-cytosine and adenine-thymine) that allow the DNA helix to maintain a regular helical structure.
Breeding population size (N_b) The number of breeders in a population. This number typically is lower than the census population size.
Demographic connectivity Linkage of populations that depends on the relative contributions to population growth rates of dispersal compared to local recruitment (that is, survival and reproduction of residents).
Dispersal The movement of individuals from their birth site to their breeding site ('natal dispersal'), as well as the movement from one breeding site to another ('breeding dispersal').
DNA extraction Process of purifying genomic DNA from a tissue or other sample. Most processes include both physical and chemical extraction steps.
Effect size The magnitude, or size, of an effect. Effect size emphasizes the size of the difference rather than confounding this with sample size and may have advantages over tests of statistical significance alone.
Effective population size (N_e) The average number of individuals in a population that contribute genes to succeeding generations. This number generally is lower than the census population size.
Fitness The net effect of viability, mating success, and fecundity that determines number of progeny.
Gene Each gene is a linear segment of a DNA molecule that includes a specific sequence of paired bases that are arranged on chromosomes. Each gene is responsible for a single inherited property, characteristic, or function of the organism.
Gene expression The appearance in a phenotype of a characteristic or effect attributed to a particular gene. An expression profile is the measure of activity (expression) of many genes at once.
Gene flow The movement of genes among populations connected by dispersal and migration. The incorporation of "migrant" genes into a population requires both successful immigration and successful interbreeding.
Gene sequencing The process of determining the precise order of nucleotides within a DNA molecule.
Genetic bottleneck A sharp reduction in the size of a population. Also called a population bottleneck.
Genetic differentiation A process in which two or more populations accumulate independent genetic changes (DNA sequence mutations) or allele frequency differences through time as a result of reduced gene flow or complete reproductive isolation for some period of time. Estimators of genetic differentiation include Wright's fixation index (F_{ST}) and Weir & Cockerham's index θ. Also called genetic divergence.

Genetic diversity The existing genetic variation within a population or species. Various measures are used to estimate genetic diversity such as determining the number of polymorphic sites across a specified region of DNA sequence or determining the number of heterozygous individuals in a population. Some commonly used genetic diversity measures include polymorphic sites(s), heterozygosity (H), and allelic richness (A_r).
Genetic drift The change in allele frequencies over time due to the chance disappearance of particular alleles as individuals die or do not reproduce. Drift is stronger in smaller populations and leads to greater genetic differentiation among populations and lower diversity within them.
Genetic recapture A technique in which scat or hair are genotyped at several loci to provide a unique individual ID that is used in a mark-recapture study.
Genetic structure The distribution of genotypes within and among populations.
Genotype A genetic profile indicating the particular alleles present at one or more loci within an organism.
Heterozygosity A diploid organism is heterozygous at a gene locus when there are two different alleles of a gene present. Heterozygosity is the (expected) probability that an individual will be heterozygous at a given locus.
High throughput sequencing See gene sequencing. High-throughput sequencing can sequence very large quantities of DNA at one time. Platforms include Illumina® HiSeq, MiSeq (Illumina, Inc.), and PacBio® RS (Pacific Biosciences of California, Inc.), among others.
Independent markers Gene regions that are scattered throughout the genome and not physically linked.
Linkage disequilibrium The non-random association of alleles at different loci. Loci are said to be in linkage disequilibrium when the frequency of association of their different alleles is higher or lower than what would be expected if the loci were independent and associated randomly. The premise of the linkage disequilibrium method to calculate N_e is that the magnitude of random association of alleles at different gene loci is determined by three variables: N_e, the number of individuals sampled (S), and the recombination rate between loci (c).
Linked genes or loci Genes or loci that are physically located close together on the same chromosome and do not sort independently.
Local adaptation The process by which organisms in a particular locality have differentially evolved as compared to other localities in response to selective pressures imposed by some biotic or abiotic aspect of their local environment.
Metapopulation A group of sub-populations that occupy spatially separated patches of habitat and are connected by some level of movement and gene flow.
Microsatellite loci Short, repeated sequences of noncoding DNA that are dispersed throughout the genome. The repeated sequence is often simple, consisting of two, three, or four nucleotides (di-, tri-, and tetra-nucleotide repeats respectively). Individuals can vary in the number of repeat units per microsatellite allele; this variability constitutes the data used in population genetic analyses. Microsatellites evolve rapidly because mutations (represented by differences in the number of repeat units) typically have no effect on the organism, making them especially useful for understanding patterns of contemporary evolution (that is, migration and gene flow). Also known as short tandem repeat loci (STRs).
Migration The physical movement of individuals from one area to another; can be seasonal.
Neutral markers Non-coding (selectively neutral) regions of DNA that do not confer high relative fitness.
Pedigree The network of an individual's parents, grandparents, offspring, siblings, and so forth.
Phenotypic traits Observable characteristics of an organism, including morphological, developmental, biochemical, and behavioral.

Population A group of related individuals in the same geographic area that freely interbreed.
Population genetic structure The accumulated genetic differences between groups of individuals that do not freely interbreed. Physical barriers to migration and limited dispersal can cause genetic structuring of populations. The amount of genetic structure is inversely related to the amount of gene flow.
Power The power or sensitivity of a hypothesis test is the probability that the test correctly rejects the null hypothesis (H_0) when the alternative hypothesis (H_1) is true. It can be equivalently thought of as the probability of accepting the alternative hypothesis (H_1) when it is true—that is, the ability of a test to detect an effect, if the effect actually exists. Power is influenced by sample size, the statistical significance criterion used, and the magnitude of the effect of interest.
Precision The variability around a measurement.
Quantitative trait A measurable phenotype that depends on the cumulative actions of many genes and the environment. These traits can vary among individuals, over a range, to produce a continuous distribution of phenotypes.
Restriction enzyme An enzyme that cuts DNA molecules at a specific sequence.
Selection Differential survival and reproduction among individuals due to heritable trait differences.
SNP – Single Nucleotide Polymorphism A single base mutation at a specific locus usually consisting of two alleles. In a diploid organism, an individual can be either homozygous for one or another allele copy, or heterozygous (containing one of each allele copy). Many SNPs can be combined to provide an individual genetic fingerprint or genotype.

Appendix A. Recent Genetic Studies Conducted in the San Diego County Management Strategic Plan Area

Table A1. Recent genetic studies conducted in the San Diego County Management Strategic Plan Area.

[Studies are categorized into two different types: (1) Population genetics— focus of study on determining the distribution of genetic diversity among populations, levels of gene flow, effective population size, etc.; (2) Phylogeography—focus of study is historical or evolutionary breaks within a species range (typically these studies survey fewer individuals across a range than population genetic studies). **Abbreviations:** mtDNA, mitochondrial deoxyribonucleic acid; HWE, Hardy-Weinberg equilibrium; RAPDs; AFLPs, amplified fragment length polymorphisms; ISSRs, inter simple sequence repeats; SNPs, single nucleotide polymorphisms]

Species	Study extent (counties)	Year(s) sampled	Marker types	Reference(s)	Major results
Coastal cactus wren (*Campylorhynchus brunneicapillus*)	U.S. range: San Diego, Orange, Riverside, San Bernadino, Los Angeles, Ventura	2011–14	microsatellites, mtDNA	Barr and others, 2015; Barr and Vandergast, 2014.; Eggert, 1996.	Population genetics: Genetic structure mirrored underlying habitat availability, with cluster and population boundaries coinciding with fragmentation caused primarily by urbanization. Within populations, there was a positive association between available local habitat and allelic richness and a negative association with relatedness. Genetic bottleneck signals were associated with wildfire frequency. Results indicate that habitat fragmentation and alterations have reduced genetic connectivity and diversity of cactus wren populations in coastal southern California. **Management:** Improving connectivity among remaining populations may help to ensure population persistence.
California gnatcatcher (*Polioptila californica)*	U.S. range: San Diego, Orange, Riverside, San Bernadino, Los Angeles, Ventura	2012–14	microsatellites	Vandergast and others, 2014.	Population genetics: Clustering analyses detected a single genetic cluster across the entire study area (Ventura–San Diego Counties). Finer scale population subdivision was detected among three more isolated aggregations in Ventura, Palos Verdes, and Chino Hills. Genetic diversity was highest in the south and decreased with increasing latitude. The effective population size of the entire cluster was greater than 1,000. **Management:** Population monitoring and trend analysis could continue and may benefit from regional coordination across San Diego, Orange, western Riverside, San Bernardino and Los Angeles Counties, as birds in these regions form a single genetic unit.

Species	Study extent (counties)	Year(s) sampled	Marker types	Reference(s)	Major results
Southern mule deer (*Odocoileus hemionus fuliginatus*)	San Diego	2015	microsatellites	Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016.	Population genetics: **Central San Diego**: Southern mule deer have less overall genetic diversity than subspecies elsewhere in the State, with an effective population size that is less than 200 individuals for the region. Mule deer are relatively sedentary/territorial over many years. Offspring often remain very close to one or both parents. Females in close proximity tend to be more closely related than males in close proximity. **67 Study:** Recaptures generally were detected close to original capture locations (within 1.5 kilometer). We did not detect recaptures across roads; however, pedigree analysis detected 21 first-order relative pairs, of which about 20 percent were detected across State Route 67. Exact tests comparing allele frequencies between groups of individuals in predefined geographic clusters detected significant genetic differentiation across State Route 67. In contrast, clustering analysis supported a single genetic cluster across the study area. **Management:** Deer form 2–9 distinct management units; State Route 67 may reduce, but does not preclude, movement and gene flow.
San Diego thornmint (*Acanthomintha ilicifolia*)	San Diego	2012–14	isozymes	Rogers, 2014.	Population genetics: Banding patterns suggested that diploid, tetraploid, hexaploid individuals occurred throughout range, some populations of mixed ploidy.
San Diego ambrosia (*Ambrosia pumila*)	San Diego, Riverside	2003	RAPDs	McGlaughlin and Friar, 2007.	Population genetics: 31 multi-locus genotypes were identified from the 201 stems sampled. The spatial distribution of clones was limited with no genotypes shared between plots or populations. Mean clone size was estimated at 9.10 ramets per genet. Genets in most plots were intermingled. The maximum genet spread was 0.59 meters, suggesting that genets can be larger than the sampled 0.25-square-meter plots. Spatial autocorrelation analysis detected a lack of spatial genetic structure at short distances and significant structure at large distances within populations. **Management:** Because of the occurrence of multiple genets within each population, the limited spread of genets, and a localized genetic structure, conservation activities could focus on the maintenance of multiple populations throughout the species range.

Species	Study extent (counties)	Year(s) sampled	Marker types	Reference(s)	Major results
San Diego fairy shrimp (*Branchinecta sandiegonensis*)	San Diego, Orange	1990s–present	allozymes, mtDNA	Davies and others, 1997; Bohonak, 2005; Vandergast and others, 2009.	Population genetics, phylogeography: Allozymes: high structure among populations, low variability within. Many loci not conforming to HWE. mtDNA: high structure, 2 major clades, mixing in areas with high disturbance. Cyst extraction and mtDNA primers for species identifications for dry season presence surveys. **Management**: Disturbance may mix previously separated gene pools.
Quino checkerspot butterfly (*Euphydryas editha quino*)	San Diego, Riverside	early 2000s	microsatellites	Miller and others, 2014.	Population genetics: Four wild and two captive populations surveyed at six microsatellite loci; medium to high-frequency alleles from wild populations also were present in the captive populations. No significant difference in genetic diversity as quantified by expected heterozygosity; the captive populations showed tendencies toward significantly lower allelic richness than their wild counterparts. **Management:** Periodic incorporation of new wild specimens into the captive population suggested to assure that allelic diversity is maintained to the extent possible.
Hermes copper butterfly (*Lycaena hermes*)	San Diego	2003–2012	AFLPs	Strahm and others, 2012.	Population genetics: Some weak structuring. Individuals from peripheral populations in the northern and western part of the Hermes copper distribution generally show increased differentiation compared to populations in the central region of their range (McGinty Mountain, Sycuan Peak, and Lawson Peak areas). The southeastern peripheral populations near Potrero appear to have adequate dispersal with the central region to prevent genetic differentiation.
Orcutt's spineflower (*Chorizanthe orcuttiana*)	San Diego, Point Loma	2001, 2005	allozymes, ISSRS	Bauder and others, 2010.	Population genetics: Low variation recovered with allozymes (three polymorphic allozyme loci); fine scale genetic mosaic, but low among site divergence, perhaps due to seed dispersal by animals.
Riverside fairy shrimp (*Streptocephalus wootoni*)	San Diego, Riverside, Orange	2010	mtDNA	Lahti and others, 2010.	Population genetics, phylogeography: Very low genetic diversity, only five mtDNA COI haplotypes recovered. All populations north of Camp Pendleton were monomorphic for the most common haplotype. Patterns suggest either a recent northward range expansion or mtDNA selective sweep. More independent loci could be examined. Amplification rates decreased with cyst storage time, suggesting that dry storage of soil may result in decreased cyst viability over time.

Species	Study extent (counties)	Year(s) sampled	Marker types	Reference(s)	Major results
Orange-throated whiptail (*Aspidoscelis hyperythra*)	San Diego	2007, 2015	microsatellites	Luckau, 2015.	Population genetics: Five replicate sites across the county. Point Loma showed low genetic diversity and was susceptible to a barrier effect of the road. Analysis of pairs of first-order relatives indicated longer-distance dispersal events than previously reported. **Management:** Point Loma population had lowest diversity and some fragmentation effects.
Pond turtle (*Emys marmorata pallida*)	Ventura, San Bernardino, Los Angeles, Orange, Riverside, Northern Baja California	1990s, 2010s	SNPs, mtDNA	Fisher and others, 2012.	Population genetics: We recovered the large genetic differences occurring in the previous studies of the turtles between northern and southern populations. We also detected evidence of pond turtles being moved into the area from the north, as released or escaped pets. The SNP data had enough resolution to identify "natural" breaks in the species, so that management units for conservation could be developed. In assessing genetic bottlenecks, we determined that only the most remote and undisturbed sites appear genetically intact.
Coastal horned lizard (*Phrynosoma blainvilli*)	San Diego	1990s–present	SNPs	Jonathan Richmond, U.S. Geological Survey, unpub. data.	Population genetics: In progress.
Western snowy plover (*Charadrius nivosus nivosus*)	Species range: Western Hemisphere	1995–2005	mtDNA, microsatellites	Funk and others, 2007.	Population genetics: Low genetic differentiation among sampled breeding sites in North America.
Clapper rail (Ridgeway's rail) (*Rallus longirostris levipes*)	San Diego, Ventura, Orange, Imperial	1989	mtDNA, RAPDs, minisatellites	Fleischer and others, 1995; Nusser and others, 1996.	Population genetics: Low genetic variation within populations, recent bottlenecks. **Management:** Captive breeding established and augmentations underway.

Species	Study extent (counties)	Year(s) sampled	Marker types	Reference(s)	Major results
Least tern *(Sternula antillarum browni)*	Species range: United States, Caribbean	Not stated	mtDNA, microsatellites	Draheim and others, 2010.	Phylogenetics, subspecies designations: Little evidence of genetic structure within the three traditional subspecific groups across the United States. No differentiation between two California breeding populations. Isolation-by-distance analyses, however, identified subtle patterns that may represent sex-specific differences in dispersal behavior. Little population subdivision among subspecific groups, which raises questions regarding the taxonomic status of traditionally defined subspecies.
Least Bell's vireo (*Vireo bellii pusillus*)	Species range: United States, Mexico	Not stated	mtDNA, SNPs	Klicka and others, 2016	Phylogenetics, subspecies designations: Phylogenetic analyses uncovered two distinct clades that are separated in the arid southwestern United States, near the border of the Chihuahuan and Sonoran Deserts. These clades diverged from each other about 1.11–2.04 million years ago. The timing of diversification, geographic location, and niche modeling of the east/west divergence suggest vicariance as a mode of diversification for these two lineages. Analyses of the SNP dataset provided additional resolution and indicated that the Least Bell’s vireo populations are a distinct evolutionary lineage. Our genetic evidence, together with information from morphology and behavior, suggests that the Bell’s vireo complex involves two species, each containing two separate subspecies. **Management**: Species and subspecies could be managed as distinct units.
Pallid bat (*Antrozous pallidus*)	Species range: California, Western United States, Mexico	not stated	mtDNA, AFLPs	Lack and others, 2010.	Phylogeography: California populations are distinctive; San Diego bats not included in analysis.

Species	Study extent (counties)	Year(s) sampled	Marker types	Reference(s)	Major results
San Diego pocket mouse (*Chaetodipus fallax fallax*)	Species range: California, Baja California	not stated	mtDNA	Rios and Álvarez-Castañeda, 2010.	Phylogenetics, subspecies designations: Genetic relationships were evaluated among the 6 recognized subspecies of *C. fallax* in 3 geographic regions from individuals representing 22 populations. Analysis of molecular variance and multiple phylogenetic analyses indicated three main clades: (1) northern populations in the southwestern Mojave Desert and Los Angeles Basin north of the Salton Trough;(2) central populations from south of the Salton Trough and throughout the state of Baja California; and (3) southern populations from west of the Vizcaino Desert in Baja California Sur and adjacent Isla Cedros. These clades do not correspond to the currently recognized subspecies.
Stephen's kangaroo rat (*Dipodomys stephensi*)	Southern California	1990s; 2008–2015	mtDNA, microsatellites	Metcalf and others, 2001; Shier and Navarro, 2016.	Population genetics: High genetic diversity across range, high differentiation. Lower diversity in southern populations, indicating a recent range expansion southward. Many distinct genetic populations detected with microsatellite markers, with an overall isolation by distance pattern. Habitat fragmentation may be driving genetic differentiation among sites. **Management**: Re-establishing gene flow among sites recommended in Shier and Navarro (2016).
Mountain lion (California puma) (*Puma concolor*)	California, Southern California	2001–2012	microsatellites	Ernest and others, 2003; Ernest and others, 2014.	Population genetics: Peninsular Range and Santa Ana Mountains populations showed relatively low connectivity to other regions. Peninsular and desert populations were connected. Santa AnaMountains population had very low effective population sizes, and two individuals with the lowest diversity also had kinked tails. **Management:** Could restore connectivity between Santa Ana mountains and Peninsular Range, particularly across Interstate 15.

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